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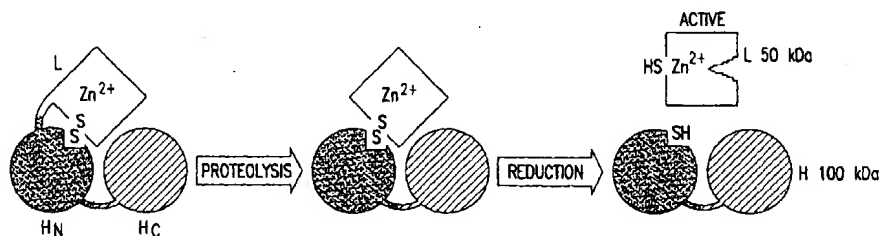
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(54) Title: FRET PROTEASE ASSAYS FOR BOTULINUM SEROTYPE A/E TOXINS



(57) Abstract: The present invention provides clostridial toxin substrates useful in assaying for the protease activity of any clostridial toxin, including botulinum toxins of all serotypes as well as tetanus toxins. A clostridial toxin substrate of the invention contains a donor fluorophore; an acceptor having an absorbance spectrum overlapping the emission spectrum of the donor fluorophore; and a clostridial toxin recognition sequence that includes a cleavage site, where the cleavage site intervenes between the donor fluorophore and the acceptor and where, under the appropriate conditions, resonance energy transfer is exhibited between the donor fluorophore and the acceptor.

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FRET PROTEASE ASSAYS FOR BOTULINUM SEROTYPE A/E TOXINSBACKGROUND OF THE INVENTIONFIELD OF THE INVENTION

The present invention relates generally to
5 fluorescence resonance energy transfer and protease
assays, for example, assays for protease activity of
clostridial toxins such botulinum toxins and tetanus
toxins, and more specifically, to intramolecularly
quenched substrates and methods for assaying for
10 clostridial toxin protease activity.

BACKGROUND INFORMATION

The neuromuscular syndrome of tetanus and the
rare but potentially fatal disease, botulism, are caused
by neurotoxins produced by bacteria of the genus
15 *Clostridium*. These clostridial neurotoxins are highly
potent and specific poisons of neural cells, with the
human lethal dose of the botulinum toxins on the order of
micrograms. Thus, the presence of even minute levels of
botulinum toxins in foodstuffs represents a public health
20 hazard that must be avoided through rigorous testing.

However, in spite of their potentially
deleterious effects, low controlled doses of botulinum
neurotoxins have been successfully used as therapeutics.
25 These toxins have been used in the therapeutic management
of a variety of focal and segmental dystonias, of
strabismus and other conditions in which a reversible
depression of a cholinergic nerve terminal activity is
desired. Established therapeutic uses of botulinum
30 neurotoxins in humans include, for example,